Impairment of systemic microvascular endothelial and smooth muscle function in individuals with early-onset coronary artery disease: studies with laser speckle contrast imaging

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Objectives **The assessment of systemic microvascular reactivity is currently considered to be critical in the stratification of cardiovascular risk. In the present study, we compared skin microvascular function in individuals with early-onset (premature) coronary artery disease (EOCAD, *n*=30) with that of age-matched and sex-matched healthy individuals (*n*=30).**

Materials and methods **Using laser speckle contrast imaging, cutaneous blood flow was assessed in the forearm at rest and during reactivity tests, including postocclusive reactive hyperemia and the iontophoresis of acetylcholine or sodium nitroprusside with increasing currents of 30, 60, 90, 120, 150, and 180 μA for 10-s intervals spaced 1 min apart. Carotid intima–media thickness was evaluated using an ultrasound system and a 7.5 MHz ultrasound transducer.**

Results **The endothelium-dependent skin microvascular vasodilator responses that were induced by both acetylcholine and postocclusive reactive hyperemia were significantly reduced in patients with EOCAD compared with healthy individuals. The vasodilator responses that were induced by sodium nitroprusside were also significantly reduced in individuals with EOCAD. These systemic microvascular alterations were concurrent with increased carotid intima–media thickness in these patients.**

Conclusion **Laser speckle contrast imaging identifies endothelial-dependent and endothelial-independent microvascular dysfunction in individuals presenting with EOCAD, and thus could be valuable as an early peripheral marker of atherothrombotic disease. *Coron Artery Dis* 25:23–28 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: carotid intima–media thickness, early-onset coronary artery disease, laser speckle, microvascular endothelial dysfunction, microvascular reactivity, microvascular smooth muscle dysfunction

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Introduction

In recent years, the assessment of systemic microvascular reactivity has proved to be essential in the investigation of the pathophysiology of cardiovascular diseases and stratification of cardiovascular risk [1,2]. In addition, the cutaneous microcirculation is now considered an accessible and representative vascular bed for the assessment of systemic microcirculatory reactivity [3–5]. Moreover, the alterations of microvascular function in the skin were shown to be correlated with increased coronary artery disease risk [6].

In this context, laser speckle contrast imaging (LSCI) provides an innovative approach for the noninvasive evaluation of systemic microvascular endothelial function [4,7,8]. A major advantage of this technique is that the reproducibility of LSCI is superior to that of earlier procedures, such as laser Doppler flowmetry and laser Doppler imaging [9,10]. Moreover, LSCI has already proved to be an effective noninvasive technique in the evaluation of systemic microvascular reactivity in patients presenting with cardiometabolic diseases [7,11].

Although most prevalent in elderly individuals, coronary heart disease also affects younger adults [12]. Early-onset (premature) coronary artery disease (EOCAD) has shown flattening trends in mortality rates, suggesting that the cardiovascular disease epidemic is not being controlled [13].

In the present study, we compared the cutaneous microvascular reactivity of healthy individuals with that of individuals with EOCAD using LSCI coupled with physiological or pharmacological local vasodilator stimuli. Endothelial microvascular function was evaluated using postocclusive reactive hyperemia (PORH) and the transdermal iontophoretic delivery of acetylcholine (ACh), whereas endothelium-independent vasodilation was studied using the iontophoresis of sodium nitroprusside (SNP), as recently comprehensively revised by

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Roustit and Cracowski [4]. Briefly, PORH-induced vasodilation is considered to be a useful index of global microvascular reactivity, at least partially dependent on endothelial function [4]. Different endothelial mediators appear to be involved in the hyperemic response that follows arterial occlusion, including nitric oxide (NO), cyclooxygenase metabolites, and endothelium-derived hyperpolarizing factor (EDHF) [4]. This is also the case for vasodilation of the microvessels resulting from ACh iontophoresis, which is clearly related to endothelial function [4]. In contrast, vasodilation that results from SNP iontophoresis, which is a direct donor of NO, is apparently independent of endothelial function [4].

Our primary aim was to test whether LSCI could identify microvascular endothelial dysfunction in patients with EOCAD. A secondary objective was to investigate whether EOCAD is characterized by microvascular smooth muscle dysfunction. Carotid intima–media thickness (CIMT), the increase of which is associated with the presence and extent of coronary artery disease [14], was also evaluated and was used as a surrogate marker of atherosclerotic cardiovascular disease.

Materials and methods

Study design

This cross-sectional study included 30 patients with EOCAD who were below 45 years of age at diagnosis for men and below 55 years of age at diagnosis for women [15] and 30 healthy age-matched and sex-matched individuals. The clinical characteristics of the patients and controls are described in Table 1. EOCAD was defined by the occurrence of any acute coronary syndrome, including ST elevation or non-ST elevation, myocardial infarction, or unstable angina (all defined by characteristic history, electrocardiographic, and cardiac enzyme abnormalities), or by the diagnosis of obstructive coronary artery disease at coronary angiography (defined as $\geq 50\%$ stenosis of any epicardial coronary artery) in patients with stable angina. Hypertension was defined as blood pressure (BP) of at least 140/90 mmHg and/or antihypertensive drug use; all hypertensive patients included in the study were under effective antihypertensive treatment and had their BP under control. Diabetes mellitus was defined by history and use of insulin or oral hypoglycemic medications, or fasting glucose levels greater than 100 mg/dl. Previous myocardial revascularization (either percutaneous or coronary artery bypass surgery) was defined by history.

The present study was undertaken in accordance with the Helsinki Declaration of 1975, as revised in 2000, and was approved by the Institutional Review Board of the National Institute of Cardiology of Rio de Janeiro, Brazil, under protocol number #0332/1105211. Once considered eligible, all participants read and signed an informed consent document approved by the IRB. For biochemical testing, venous blood samples were obtained in the morning after 12 h of fasting and microcirculatory tests

Table 1 Clinical characteristics of healthy controls and of individuals with early-onset coronary artery disease

Characteristics	Healthy individuals (n=30)	EOCAD (n=30)	P value
Age (years)	42.1±0.6	42.6±0.5	0.4174
Male [n (%)]	15 (50)	18 (60)	0.6042
Smokers [n (%)]	6 (20)	9 (30)	0.5520
Diabetes [n (%)]	0 (0)	9 (33)	**0.0088**
Hypertension [n (%)]	1 (3)	27 (90)	**<0.0001**
Dyslipidemia [n (%)]	0 (0)	26 (87)	**<0.0001**
Family history of myocardial infarction [n (%)]	1 (3)	25 (83)	**<0.0001**
Acute myocardial infarction [n (%)]	0 (0)	26 (87)	**<0.0001**
Atherosclerotic plaques in carotid arteries [n (%)]	2 (7)	6 (20)	0.2542
Coronary bypass surgery [n (%)]	0 (0)	8 (27)	**0.0046**
Coronary angioplasty [n (%)]	0 (0)	20 (67)	**<0.0001**
Weight (kg)	73.8±2.9	89.0±4.3	**0.0026**
Height (cm)	168.1±2.1	164.2±1.9	0.1807
BMI (kg/m^2)	26.1±0.7	32.7±1.1	**<0.0001**
Waist circumference (cm)	89.4±2.1	105.5±3.2	**<0.0001**
Systolic blood pressure (mmHg)	130.0±3.4	131.7±5.0	0.7844
Diastolic blood pressure (mmHg)	85.1±2.2	84.6±3.4	0.9027
Mean blood pressure (mmHg)	99.5±2.6	101.0±3.7	0.8007
Creatinine (mg/dl)	0.81±0.03	0.85±0.04	0.3312
Urea (mg/dl)	28.3±2.4	31.1±1.7	0.3462
Total cholesterol (mg/dl)	201.0±7.1	202.3±11	0.5258
Triglycerides (mg/dl)	76.5 (62–117)	157 (110–265)	**<0.0001**
HDL-C (mg/dl)	48.2±2.5	36.8±1.3	**0.0003**
LDL-C (mg/dl)	142.5 (102–152)	114 (90–140)	0.1554
Glucose (mg/dl)	92.5 (88–97)	105 (96–128)	**0.0004**

The results were presented as mean±SEM. For values that did not follow a Gaussian distribution, the medians (25th–75th percentile) were presented (Shapiro–Wilk normality test).
All hypertensive patients included in the study were under effective antihypertensive treatment and had their blood pressure under control.
EOCAD, early-onset coronary artery disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Bold values represent statistically significant differences.
P values were estimated using two-tailed unpaired Student's t tests, Mann–Whitney tests or Fisher's exact tests, as appropriate.

were performed after a 20-min rest in the supine position in a temperature-controlled room (23±1°C). The patients took their usual medication on the morning of the tests. The brachial systolic (SAP) and diastolic (DAP) BPs were measured twice, 1 min apart, immediately before the beginning of the recordings, using a mercury sphygmomanometer, and the mean values were recorded as the patients' clinical BP. The mean arterial pressure was calculated as DAP + 1/3(SAP – DAP).

Evaluation of microvascular reactivity

Microvascular reactivity was evaluated using an LSCI system with a laser wavelength of 785 nm (PeriCam PSI system; Perimed, Järfälla, Sweden) in combination with the iontophoresis of ACh or SNP for the noninvasive and continuous measurement of cutaneous microvascular perfusion changes measured in arbitrary perfusion units (APU) (Fig. 1). The images were analyzed using the manufacturer's software (PIMSoft; Perimed). Two skin sites on the ventral surface of the forearm, ~5 cm apart, were randomly chosen. Hair, broken skin, areas of skin pigmentation, and visible veins were avoided and two drug-delivery electrodes were installed by means of adhesive discs (LI 611; Perimed). Three measurement areas were determined; two of the measurement areas were within the electrodes (ACh and SNP), and the third (PORH) was adjacent to the electrodes. A vacuum cushion (AB Germa, Kristianstad, Sweden) was used to reduce recording artifacts generated by arm movements. ACh 2% w/v or SNP 2% w/v (Sigma Chemical Co., St Louis, Missouri, USA) iontophoresis was performed using a micropharmacology system (PF 751 PeriIont USB Power Supply; Perimed) with increasing anodal (ACh) or cathodal (SNP) currents of 30, 60, 90, 120, 150, and 180 μA for 10-s intervals spaced 1 min apart. The total charges were 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mC, respectively. The dispersive electrode was attached ~15 cm away from the iontophoresis chamber. The results of the pharmacological tests were expressed both as peak values, representing the maximal vasodilation observed after the highest dose of ACh or SNP, and area under the curve of vasodilation (Fig. 1b). The use of the area under the blood flow/time curve for assessing skin microvascular reactivity using laser technology is well validated because it represents the global flow response to different physiological and pharmacological stimuli [4].

During the PORH test, arterial occlusion was performed with suprasystolic pressure, defined as 50 mmHg above SAP, by applying a sphygmomanometer for 3 min [16]. Following the release of pressure, the maximum flux was measured. The measurements of skin blood flux were divided by mean arterial pressure to give the cutaneous vascular conductance (CVC) in APU/mmHg. The amplitude of the PORH responses was expressed as the peak CVC minus the baseline CVC. CIMT was evaluated according to the guidelines of the American Society of Echocardiography [17] using a Vivid S6 ultrasound system and a 7.5 MHz ultrasound transducer (GE Healthcare, Waukesha, Wisconsin, USA). The PORH tests were always performed after iontophoresis of ACh and SNP to avoid interference with these pharmacological tests, which are limited to local responses in the skin. In contrast, the hyperemic response is a global one that extends to the skin of the whole forearm and thus could interfere with subsequent tests of microvascular reactivity.

Statistical analysis

The results were presented as mean±SEM. For values that did not follow a Gaussian distribution, the medians (25th–75th percentile) are presented (Shapiro–Wilk normality test). The results were analyzed using two-tailed unpaired Student's *t* tests or Mann–Whitney tests, respectively. The contingency tables were analyzed using Fisher's exact test. *P* values less than 0.05 were considered statistically significant.

Results

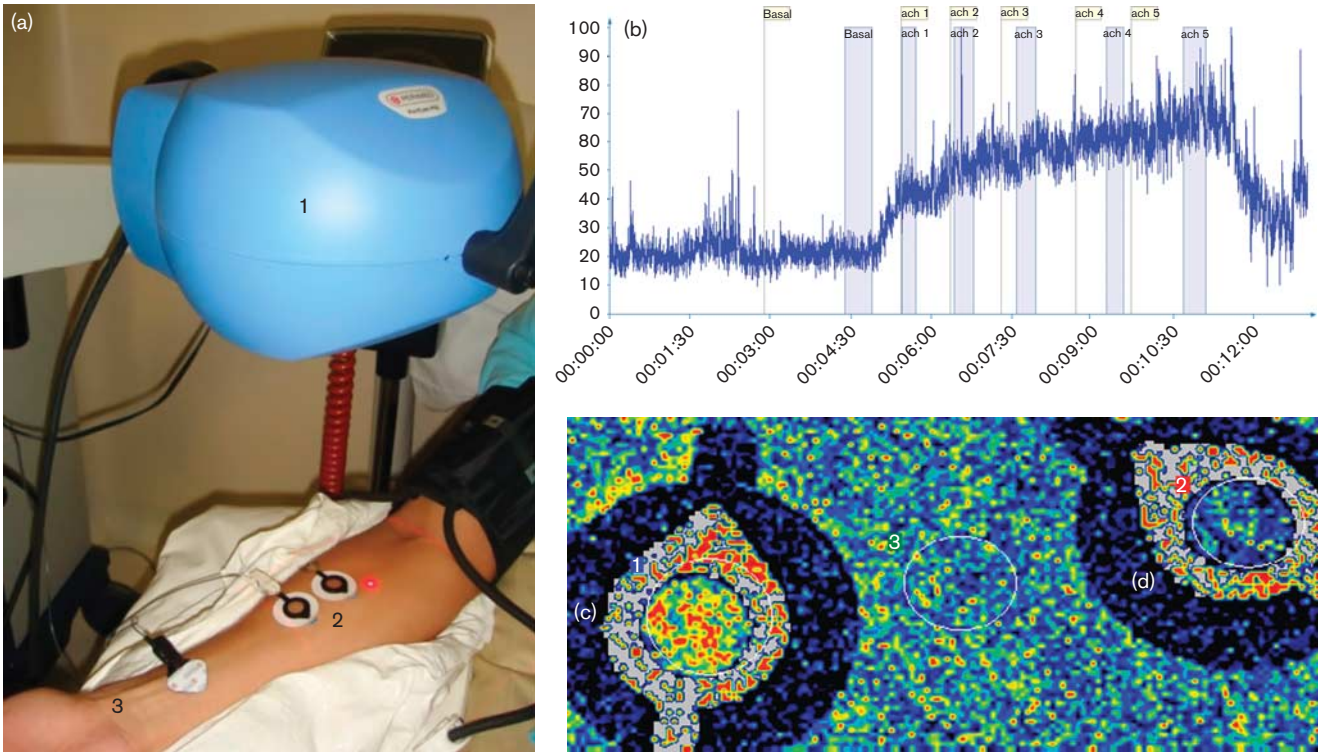
The peak values of CVC during iontophoresis of ACh were 0.61±0.04 and 0.48±0.03 APU/mmHg in the control and EOCAD participants, respectively ($P = 0.0119$; Fig. 2). The area under the curve for ACh-induced vasodilation was 7258±730 and 3723±789 APU/s in the control and EOCAD participants, respectively ($P = 0.0025$; Fig. 2). The increase in CVC resulting from iontophoresis of ACh was 0.39±0.03 and 0.26±0.0 APU/mmHg in the control and EOCAD participants, respectively ($P = 0.0023$; Fig. 2). The increase in CVC resulting from PORH-induced vasodilation was 0.48±0.03 and 0.39±0.02 APU/mmHg in the control and EOCAD participants, respectively ($P = 0.0239$; Fig. 2).

The peak values of CVC during iontophoresis of SNP were 0.51 (0.39–0.67) and 0.38 (0.29–0.49) APU/mmHg in the control and EOCAD participants, respectively ($P = 0.0159$; Fig. 3). The increase in CVC resulting from iontophoresis of SNP was 0.33 (0.03–0.67) and 0.18 (0–0.57) APU/mmHg in the control and EOCAD participants, respectively ($P = 0.0169$; Fig. 3). The area under the curve for SNP-induced vasodilation was 3225 (1549–6933) and 0 (0–2058) APU/s in the control and EOCAD participants, respectively ($P = 0.0006$; Fig. 3). In contrast, there was an increase in CIMT in the EOCAD individuals. Specifically, the CIMT was 0.8 (0.8–0.8) and 0.9 (0.8–1.0) mm in the control and EOCAD participants, respectively ($P = 0.0003$; Fig. 3).

Conclusion

The main findings of this study are as follows: (i) the endothelium-dependent skin microvascular vasodilator responses are significantly reduced in patients with EOCAD compared with healthy individuals; (ii) the microvascular smooth muscle-dependent vasodilator responses are also reduced in patients with EOCAD; and (iii) these microvascular alterations are concurrent with an increase in CIMT in EOCAD patients.

Fig. 1



Experimental set-up used in the assessment of skin microvascular perfusion using laser speckle contrast imaging coupled with iontophoresis of vasodilator drugs (a). Representative example of the effects of the transdermal iontophoretic delivery of cumulative doses of acetylcholine on skin blood flow (b) and video images of the acetylcholine iontophoresis (c) compared with a vehicle-containing electrode (d). (1) Imager head, (2) drug-delivery iontophoresis electrodes, (3) dispersive electrode (see the Materials and methods section).

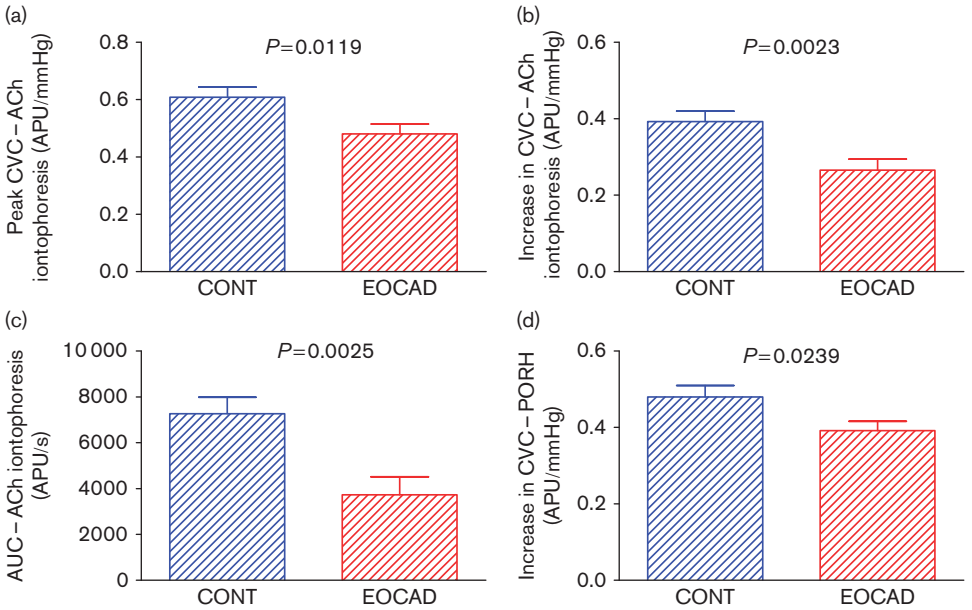
It has been previously demonstrated that in young individuals with a history of myocardial infarction, structural (carotid artery) and functional (brachial artery) alterations of the macrocirculation are already present at an early age [18]. In the present study, we showed that, similarly, systemic microvascular endothelial function is impaired in individuals with EOCAD. In fact, the simultaneous and significant reductions of microvascular vasodilation induced by both ACh iontophoresis and PORH, observed in our study, clearly indicate the presence of endothelial dysfunction, as previously demonstrated [4,19,20]. Most importantly, altered skin PORH has recently been shown to be an independent marker of atherosclerotic damage in patients with type 1 diabetes [21].

PORH refers to the marked increase in skin blood flow that follows the release of transitory arterial occlusion. The mechanisms underlying skin microvascular vasodilation during PORH are still a matter of debate [4]. In addition to classical endothelial mediators such as NO and prostaglandins, large-conductance calcium-activated potassium (BK_{Ca}) channels appear to be involved, suggesting the participation of EDHF in this response [22]. These latter results indirectly suggest the involvement of epoxyeicosatrienoic acids, which are cytochrome P-450 epoxygenase metabolites of phospholipase-dependent arachidonic acid production [23].

A variety of endothelium-derived vasodilator mediators also appear to be released following ACh skin iontophoresis, including EDHF, NO, and prostaglandins [4,24–26]. Notwithstanding, even if the mechanisms involved in microvascular vasodilation induced by physiological (PORH) and pharmacological (iontophoresis of ACh) tests of microvascular reactivity are multiple, complex, and interdependent, both tests are well known to reflect endothelial function [4,27–29].

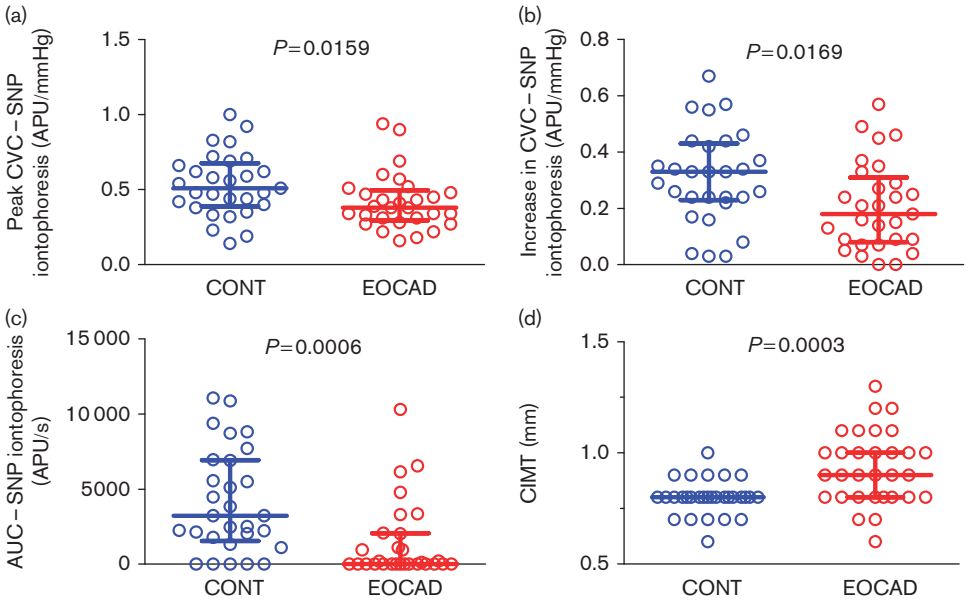
We also observed significant endothelial-independent alterations in microvascular reactivity in patients with EOCAD, indicating impaired smooth muscle function. This could be due to reduced sensitivity of the NO/cGMP pathway, considering that SNP is a direct donor of NO, which skirts the endothelium and directly relaxes the adjacent vascular smooth muscle cells. In addition, altered endothelium-independent vasodilation observed in patients with EOCAD could be related to structural alterations of the microvessels (arteriolar remodeling), as these alterations are concurrent with an increase in CIMT in these patients, when compared with age-matched and sex-matched individuals.

Fig. 2



The peak effects of skin iontophoresis of acetylcholine (ACh) on cutaneous microvascular conductance [CVC, expressed in arbitrary perfusion units (APU) divided by mean arterial pressure in mmHg] of healthy volunteers (CONT) or individuals with early-onset coronary artery disease (EOCAD, a) and increase in CVC induced by iontophoresis of ACh (b). The area under the curve (AUC) of skin iontophoresis of ACh (c) and increase in CVC induced by postocclusive reactive hyperemia (PORH) in healthy volunteers and patients (d). The amplitudes of ACh and PORH responses are expressed as peak CVC minus the baseline CVC. The values are mean±SEM and were analyzed using two-tailed unpaired Student's *t* tests.

Fig. 3



The peak effects of skin iontophoresis of sodium nitroprusside (SNP) on cutaneous microvascular conductance [CVC, expressed in arbitrary perfusion units (APU) divided by mean arterial pressure in mmHg] of healthy volunteers (CONT) or individuals with early-onset coronary artery disease (EOCAD, a) and increase in CVC induced by iontophoresis of SNP (b). The area under the curve (AUC) of skin iontophoresis of SNP (c) and carotid intima–media thickness (CIMT) in healthy volunteers and patients (d). The amplitudes of SNP responses are expressed as peak CVC minus the baseline CVC. The values are the medians (25th–75th percentile) and were analyzed using two-tailed Mann–Whitney tests.

It is also worth mentioning that the group of EOCAD patients presented with higher BMIs and waist circumferences, as well as higher levels of triglycerides and lower levels of HDL-cholesterol than the control individuals. Moreover, ~30% of the patients with EOCAD presented with type 2 diabetes. These clinical features are indicative of metabolic syndrome, which is known to alter microvascular endothelial function [30]. In contrast, endothelial-independent microvascular reactivity has been shown to be normal in both obese individuals and patients with metabolic syndrome. This indicates that the reduced microvascular smooth muscle function observed in the present study does not result from the association of cardiovascular risk factors that characterize the metabolic syndrome [31,32].

In conclusion, LSCI identifies endothelial-dependent and endothelial-independent microvascular dysfunction in individuals presenting with EOCAD, and thus could be valuable as an early peripheral marker of atherothrombotic disease.

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Conflicts of interest

There are no conflicts of interest.

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